

**Development to diapause in a population of *Euphydryas gillettii* (Nymphalidae):
effects of egg cluster size and habitat parameters**

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Abstract:

I studied survivorship from the period of egg laying to winter diapause in a population of *E. gillettii* (Nymphalidae). I investigated if fitness, in the context of hatching and successful development to diapause was related to egg cluster size, habitat parameters, or both, and if there were factors affecting egg cluster size. Results indicate that hatching is affected by sun exposure of the egg cluster and predation risk and soil condition of the host plant. Egg cluster size had no effect on hatching. This suggests that there is no optimal cluster size that increases fitness of the eggs; clusters are susceptible to equal amounts of mortality regardless of their size. Post-hatching fitness remains to be determined. Egg cluster size was related to its sun exposure and the number of egg layers in the cluster. Faster development occurred for egg masses laid in one layer on plants with a low predation risk and dry soil conditions.

Introduction:

The advantage of egg clustering by butterflies has been documented (Stamp 1981, Clark et al. 1998). In general, egg clustering may reduce the possibility of desiccation, exposure to parasitoids, and the incubation period (Stamp 1980). From the females' perspective egg clustering is advantageous when larval hosts are patchily distributed and the adult population is sparse (Stamp 1980); under such conditions clustering may allow females to allocate more time to reproduction rather than searching for larval hosts. Egg clustering, therefore, results in monophagy of the best available plant and high realized fecundity (Courtney 1984).

From the larval perspective clustering may be advantageous because it may lead to aggregation in the young larval stages. The trade-offs of aggregation may be related to egg cluster size. Beneficial effects of aggregation, such as facilitated feeding among larva and reduced mortality rates may favor

larger eggs clusters. Clustering too many eggs, however, can also be harmful as a result of an early consumption of host plant tissue (defoliation) by aggregated larvae (see introduction by Matsumoto et al 1993). This suggests that perhaps there is an optimal egg cluster size that increases the survivorship of the progeny. Likewise, different stages in larval development, such as survival to diapause may depend on the length of the growing season and the food plant quality at the end of the growing season (Holdren et al. 1981). Factors such as poor nitrogen/water content and deteriorating food quality at the end growing season may prevent larvae from reaching diapause (Holdren et al. 1981). Defoliation may affect successful development, in addition to effects on floral and seed output of the host plant and availability of the host plant for egg laying in the future (Stamp 1984). Micro-climate may also affect hatching and development (Williams 1981, Weiss et al. 1988). There is evidence that female butterflies vary egg cluster size with respect to habitat conditions (Matsumoto et al. 1993).

In this study I investigate survivorship from the period of egg laying to winter diapause in a population of *Euphydryas gilletti* (Nymphalidae), a checkerspot butterfly. To date, larval development of several species, including *E. gillettii*, has been studied. Larval development in the context of clutch size or individual fate, however, has not been observed. The objectives are to investigate 1) if fitness, in the context of hatching and survival to diapause, is related to egg clusters of different sizes, i.e., is there an optimal egg cluster size whereby the fitness of *E. gillettii* eggs and larva is increased? 2) if fitness is primarily influenced by habitat parameters and 3) if there are factors affecting how many eggs are laid per cluster and 4) if hatching is a good predictor of successful development to diapause.

Methods:

1.1 Study organism

Euphydryas gillettii (Nymphalidae) is a montane butterfly found naturally in isolated regions of the northern and central Rocky Mountains. Populations occur in moist, marshy, open montane meadows, many of which have a history of disturbance (Williams 1988).

E. gillettii is considered monophagous for *Lonicera involucrata* (Caprifoliaceae) but females ovipositing and larvae feeding in *Valeriana occidentalis* (Valerianaceae) has been reported (Williams et al. 1987). Females lay their eggs in clusters and the number of egg clusters laid per host plant varies (Boggs and Ehrlich unpubl. data). Females appear to be selective in their choice of leaf for oviposition (Williams 1984) i.e., oviposition occurs on the underside of uppermost leaves predominantly facing east so that eggs masses are maximally warmed by the sun (Williams 1981).

The flight season lasts from late June to late July although it varies from year to year (C. Boggs pers. comm.). Hatching overlaps the end of the flight season. During post-hatching development larvae consume host plant tissue by forming communal feeding webs. Larvae develop to fourth instars prior to entering winter diapause inside their feeding webs.

1.2 Egg clusters and habitat parameters

The study sites are located adjacent to the Rocky Mountain Biological Laboratory (RMBL) in Gothic, Gunnison Co., Colorado, USA (2900 m, 9500 ft). The sites are moist, marshy meadows bordering the eastern slopes of Gothic mountain and the East River. Relevant to *E. gillettii*, these meadows contain spruce, willows, nectar plants, *L. involucrata* and are characterized by a history of disturbance (avalanches). The population under study was established as a result a transplant experiment from a Wyoming population (Holdren et al. 1981).

Given the high rates of mortality for eggs and prediapause larvae, 28% in 2002 (Boggs and Ehrlich unpubl. data), I observed approximately one-hundred ninety clusters on 72 plants; clusters average 198 eggs (Boggs and Ehrlich unpubl. data). The sites were surveyed haphazardly for *L. involucrata* with egg clusters. I did not select for plants of certain sizes, plants with a determined number of egg clusters, or plants in certain micro-habitats. Variation of these factors is solely a result

of females selecting were to lay their eggs; not every plant I searched had egg clusters. Furthermore, I did not select for leaves with a certain number of egg clusters nor did I select for clusters of variable sizes. Again, any variation achieved was natural and not selected for. Plants with egg clusters were visited periodically to determine if new egg clusters were laid. I searched for egg clusters until the end of the flight season; thereafter I visited all the plants to assure that I had accounted for all egg clusters laid in the plant.

Upon finding an egg cluster I recorded its color, the orientation of leaf, sun exposure, and the number of egg layers. Regarding the plant with the egg cluster, I recorded plant volume, egg cluster density (ECD) or the number of eggs per plant volume, the number of egg clusters per plant (ECP), soil wetness, and predation risk (see below). Furthermore, to measure defoliation by pre-diapause larvae I recorded the number of meristems and leaves on the branch with the egg cluster, and adjacent branches (within 15 cm). I determined egg cluster size (ECS), the number of eggs in a cluster, and number of first instars using digital photography. Eggs and first instars were counted using a computer program "Paint". For egg clusters laid in more than one layer I made the assumption that the number of eggs in the above layer (the visible eggs) is representative of the eggs not visible in the layer below, i.e., for an egg cluster laid in three layers number of eggs would equal to number of eggs in the third layer x 3 + number of visible eggs in the second layer x 2 + number of eggs visible in the first layer. I monitored egg coloration for the purpose of estimating date of hatching and to determine number of days to hatching.

I also examined if certain sun exposures resulted in faster development. Egg coloration progressed from bright yellow (recently laid eggs) to dark red to black prior to hatching. Sun exposure of individual egg clusters rather than the host plant was divided in the eight categories (1= Full AM only, 2=Full PM only, 3=AM/PM, 4= Partial AM only, 5=Partial PM only, 6=Partial AM/ Full PM, 7=Full AM/ Partial PM). Size of plant for the purposes of determining ECD was assessed by volume and predation risk i.e., by deer and elk, was ranked on scale of 1-3; three being high and one being low

predation risk. Host plant moisture (soil wetness) was also ranked on a 1-3 scale; one being dry, two being moist and three standing water. I placed the condition of the plant into three categories: healthy (very few or no dead branches, no yellow leaves); moderately healthy (some dead branches, some yellow leaves), and unhealthy (many dead branches).

Because of the nature of communal feeding webs formed by *E. gillettii* assessing actual larvae that reach diapause is difficult. Thus, larval survivorship, or number of larva that reach diapause per number of eggs that hatched, was assessed indirectly using third instars (see Williams et al. 1984 for characteristics of instars). We are currently experimenting with mark-release-recapture (MRR) of third instars using fluorescent dyes. We hope to use MRR to estimate the number of larvae that reach winter diapause.

I used an ANCOVA (see results for co-variates) to determine if ECS, percent hatching, and survivorship (S) were affected by their respective factors. ECD and ECP distributions were used to address the question of egg laying being random.

Results:

Egg cluster size (ECS) is normally distributed (fig 1a). On average approximately 125 eggs are laid per egg mass with a standard deviation of about 50 eggs. Egg clusters per plant (ECP) do not appear to be randomly distributed (fig 1b); visual inspection of the graph reveals that most plants have less than ten egg clusters. Mean ECP is about 3.22 with a standard deviation of 3.44. The distribution of ECP did not fit an expected random distribution (Poisson, logNormal). This suggests that some plants are preferred over others. The basis of preference remains to be determined. In contrast, egg cluster density (ECD) is randomly distributed (lognormal; fig. 1c). Plant volume appears to have no influence on where a female will oviposit.

Factors other than plant volume may explain the plant preference suggested by the ECP distribution. After removing non-significant factors i.e, volume of the plant, predation risk, soil

wetness, and condition of the plant, the only factor that affected ECP was sun exposure (Stepwise-ANCOVA $P=.0157$). The majority of the egg masses were exposed to morning sun of some degree, laid in one or two layers, and present in healthy plants under moist soil conditions (table 1).

Sun Exposure	Layers	Plant Condition	SW of Host Plant
AM (41)	One (79)	Healthy (174)	Moist (123)
PM (1)	Two (101)	Mod. Healthy (11)	Dry (67)
AM + PM (123)	Three (8)	Unhealthy (5)	
Part. AM (1)			
Part. AM + PM (5)			
AM + part. PM (18)			
Part. AM + part. PM (1)			

Table 1. Distribution of egg masses. Numbers in () indicate number of egg masses (N=190); SW=Soil Wetness.

ECS is related to the number of layers in the cluster (ANCOVA $P<.0001$). In particular, the ECS of eggs laid in one layer is significantly smaller compared to clusters laid in two or three layers (Fig 2a; Tukey HSD test of least square means differences, $\alpha=.05$ $q=2.365$); on average egg clusters laid in one layer are have at least 26% less eggs. After removing non-significant factors, I found a relation between ECS and sun exposure (Fig. 2b). Hence, ECS is only affected by the number of layers in the cluster and its sun exposure. ECS is not significantly different in plants of variable volumes, plants under different soil moisture conditions and plants facing different predation risks.

To date (August 26, 2004), 56% of the egg masses have hatched ($n=107/190$); all of these received morning sun (partial or full). Out of all the egg masses that have hatched, a higher proportion (relative to their counterparts) of these were laid in two layers and found on plants with moist soil conditions. Percent hatching is normally distributed with an average of 0.523 of eggs hatching per mass and standard deviation of 0.176.

Hatching is not affected by the size of the egg cluster, layers, sun exposure, number of days to hatching, predation risk, soil wetness, or condition of the host plant (ANCOVA P -values $>.0551$).

However, after, I eliminated the major non-significant factors I found a relation between hatching and sun exposure of the egg cluster, predation risk and the soil condition of the host plant (Stepwise-ANCOVA; Fig. 3). This suggests that a greater proportion of eggs hatch if they are exposed to morning and afternoon sun. This result, however, should be taken cautiously because the sample size is naturally biased towards egg masses receiving morning and afternoon sun (see table 1).

The only factors affecting egg cluster development were the number of layers in the cluster, predation risk and soil wetness of the host plant (Fig. 4a,b,c). On average it took approximately 26 days for eggs to progress from yellow (just after oviposition) to the day of first hatching (n=47); in previous years development took about 24 days (n=10, unpubl. data 1981-1987).

At this point survival rates prior to and after hatching cannot be compared. Based on hatching data, number of eggs lost in a cluster (pre-hatching mortality) is around 48%.

Discussion:

The variation in egg cluster size follows a normal distribution with a mean lower than that calculated by Boggs and Ehrlich (unpubl. data). The distribution partially fulfills predictions of the optimal egg cluster size hypothesis. I did not find evidence of an optimal cluster size that increases the fitness of the larvae (fig. 5). I have only examine hatching. Whether ECS affects survivorship after hatching remains to be seen.

The only factor that made a difference regarding the size of the egg cluster was layering and sun exposure. Clusters laid in one layer were significantly smaller (Fig 2a) and clusters exposed to morning sun/partial afternoon sun are significantly different in size (Fig. 2b). The majority of the clusters were laid in one or two layers; clusters laid in three layers were rare. This is interesting because one would expect three layers to be preferable, i.e., in terms of protection against predation and incubation of the eggs in lower layers. Clusters laid in more than one layer, however, could also be more susceptible to dislodgement.

Females did not vary egg cluster size with respect to the factors tested (Fig 2b). This suggests that females are not varying egg cluster size by making decisions after gathering information of the host plant. Matsumoto et al. (1993) have reported females varying egg cluster size in relation to larval food abundance. The variation observed in egg cluster size could be genetically determined. Egg cluster size could be related to a female's age and genetic condition. Future studies should aim a scenario in which the condition of the female laying the eggs is known. Healthier younger females may be laying more eggs or ECS could be dependent on how many clusters a female has laid, weather conditions and the date in the flight season.

Hatching was only affected by sun exposure, predation risk and soil wetness of the host plant (fig. 3). This implies that clusters were not susceptible to the same amounts of mortality. Hence, some of these factors appear to enhance survivorship to hatching. The fact that there is a relation between hatching and dry soil conditions is interesting because, the number of egg masses found in plants with dry soil conditions is much smaller (table 1). Egg masses under dryer conditions may be less susceptible to mortality because they develop faster (see fig. 4c)..The fact that egg cluster size had no impact on hatching implies that egg clusters have equal fitness regardless of size. I believe this will not apply to fitness in post-hatching period because larvae form communal feeding webs. Egg masses of small size would result in smaller aggregations of larva. Smaller aggregations of larva may be less able to form stable feeding webs were they can over winter. Egg clusters of optimal size or a size larger than optimal would probably have higher fitness during post-hatching development because of the ability of their corresponding larval aggregations to form stable feeding webs.

Previous studies have documented the effects of microclimate on egg and larval development (Williams 1981, Clark et al 1998, Weiss et al. 1988). I did not find evidence of microclimate affecting egg development. Eggs masses did not develop faster under certain sun regimes. This finding is contrary to findings by Williams 1981. This result, however, should be taken cautiously because the sample size was small (n=56). Egg masses laid in one layer and on plants with dry soil conditions and

low predation risk developed faster (fig. 4a,b,c). This suggests a trade-off in layering. While layering can reduce predation rates, by protecting lower layers, it can also slow the development of the eggs because it may prevent lower layers from receiving any sun. Compared to previous years, egg development time was longer during this season by about two days. This may be due to a colder summer than usual. Slower development may increase mortality of egg clusters, i.e., some eggs masses may not be able to hatch before the onset of colder weather.

The only factors that influence where egg masses were found is sun exposure. Most of the egg masses when found on plant in sunlit areas. This suggests that female *E. gillettii* are selective for sunlit plants at the time of oviposition.

Several questions remain unanswered. Why are females limiting oviposition sites to *Lonicera* if habitat parameters do not affect larval fitness? How might females be selecting oviposition sites? Based on casual observations of other plants at the study sites, none of these are similar in appearance to *Lonicera*. The unique shape and size of *Lonicera* leaves along with the abundance of *Lonicera* may explain why *E. gillettii* females do not oviposit elsewhere. Future studies should compare the characteristics of plants with and without egg masses. If females indeed select where to lay their eggs, they might do so by the position of the sun just prior to oviposition. For example, they may be choosing leaves that are perpendicular to the sun before beginning to oviposit. Likewise they may be choosing oviposition sites based on the size of the leaf, i.e., by choosing leaves similar to body size a female might have a better handle on the leaf at the time of oviposition.

In conclusion, a high degree of mortality occurs prior to hatching in the population studied. Nearly half of the eggs in a cluster do not hatch. Possible reasons for high mortality include heavy predation and heavy dislodgment. Due to time constraints these factors were not considered in this study.

References:

Clark B.R. and Faeth S.H. (1998). The evolution of egg clustering in butterflies: A test of the egg desiccation hypothesis. *Evolutionary Ecology* 12 (5): 543-552

Courtney, S.P. 1984. The Evolution of Egg Clustering by Butterflies and Other Insects. *The American Naturalist* 123(2): 276-281.

Holdren C.E. and Ehrlich P.R. 1981. Long Range Dispersal in Checkerspot Butterflies: Transplant Experiment with *Euphydryas gillettii*. *Oecologia* 50:125-129.

Matsumoto K., Ito, F., and Tsubaki, Y. 1993. Egg cluster size variation in relation to the larval food abundance in *Luehdorfia puziloi* (Lepidoptera: Papilionidae). *Res. Population Ecology* 35: 325-333.

Stamp, N.E. 1980. Egg Deposition Patterns in Butterflies: Why do some species cluster their eggs rather than deposit them singly? *The American Naturalist* 115(3): 367-380.

----- 1981. Effect of group size on parasitism in a natural population of the Baltimore checkerspot *Euphydryas phaeton*. *Oecologia* 49(1): 201-206.

----- (1984). Effect of defoliation by checkerspot caterpillars (*Euphydryas phaeton* and sawfly larvae (*Macrophya nigra* and *Tenthredo grandis*) on their host plants (*Chelone* spp.). *Oecologia* 63: 275-280.

Weiss, S. B., Murphy, D.D and White, R. R. (1988). Sun, Slope and butterflies: topographic determinants of habitat quality for *Euphydryas editha* *Ecology* 69: 1486-96

Williams, E.H. and Bowers M.D. 1987. Factors Affecting Host Plant Use by the Montana Butterfly *Euphydryas gillettii* (Nymphalidae). *The American Midland Naturalist* 118(1): 153-161.

Williams, E.H., Holdren C.E., and Ehrlich P.R. 1984. The Life History and Ecology of *Euphydryas Gillettii* Barnes (Nymphalidae). *Journal of the Lepidopterists' Society* 38(1): 1-12.

Williams, E.H. 1988. Habitat and Range of *Euphydryas gillettii* (Nymphalidae). *Journal of the Lepidopterists'* 42(1): 37-45.

----- 1981. Thermal Influences on Oviposition in the Montane Butterfly *Euphydryas gillettii*. *Oecologia* 50: 342-346.

Appendix:

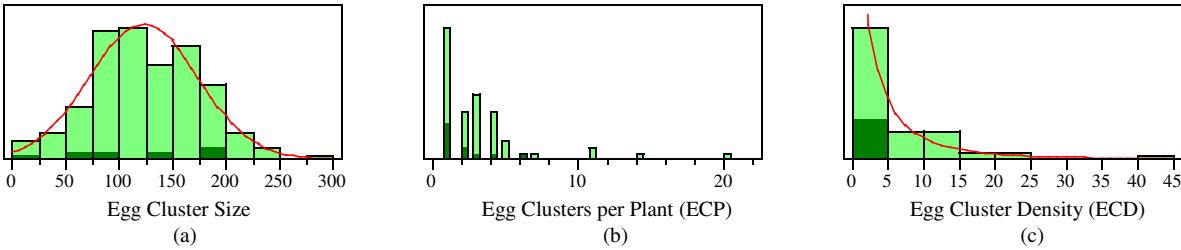


Figure 1. Distrubutions of ECS, ECP, and ECD. ECS fits a normal distribution (a), ECP did not fit a known distribution (b), and ECD fit a lognormal distribution (c).

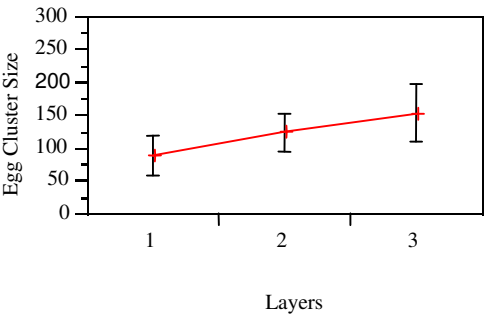


Figure 2a. Quantile plot after ANCOVA analysis with non-significant factors (see fig. 2b) included. Egg cluster size is related to the number of layers. Clusters laid in one layer are significantly smaller.

Effect	DF	F Ratio	Prob > F
Sun Exp.{2&6&8&3&4&1-7}	1	5.1131	0.0250
Sun Exp.{2-6&8&3&4&1}	1	2.7008	0.1021
Layering{1-2&3}	1	25.9690	<.0001
Layering{2-3}	1	3.9682	0.0479
N	181		

Fig 2b. Stepwise ANCOVA of ECS after removing non-significant factors: plant volume, predation risk, plant condition, and soil wetness. Egg masses laid in one layer are significantly smaller. Furthermore, egg masses with morning sun exposure/partial afternoon sun exposure (7) are significantly different in size (no quantile plot not available). Sun exposure: 1= Full AM only, 2=Full PM only, 3=AM/PM, 4= Partial AM only, 5=Partial PM only, 6=Partial AM/ Full PM, 7=Full AM/ Partial PM.

Effect	DF	F Ratio	Prob > F
Sun Exposure 2{3-1}	1	5.9674	0.0163
Predation Risk 2{2&1-3}	1	5.4025	0.0221
Soil Wetness 2	1	4.3795	0.0388
N	107		

Fig. 3. Stepwise ANCOVA of % Hatching after removing the non-significant factors: Sun Exposure 2{3&1&7-6&4}, Sun Exposure 2{3&1-7}, egg cluster size, layering, plant condition and days to hatching. Hatching is affected by sun exposure, the predation risk and soil conditions of the host plant.

Effect	DF	F Ratio	Prob > F
Layers 2{1-2&3}	1	10.1124	0.0028
Predation Risk 2{1-2&3}	1	17.3827	0.0001
Predation Risk 2{2-3}	1	1.7992	0.1870
Soil Wetness 2 (dry)	1	13.8393	0.0006
N	46		

Figure 4a. Stepwise ANCOVA of days to development after removing non-significant factors: egg cluster size, sun exposure, and plant condition. Days to development is affect by layering, low predation risk and dry soil conditions of the host plant.

Effect	DF	F Ratio	Prob > F
ECS 4	1	0.8850	0.3533
Sun Exposure 2	4	1.1426	0.3527
Layers 4	2	3.8662	0.0304
Predation Risk 3	2	10.4919	0.0003
Soil Wetness	1	17.9017	0.0002

Figure 4b. ANCOVA of days to hatching with covariates after removing plant condition days to hatching is still affected by the same factors (see below).

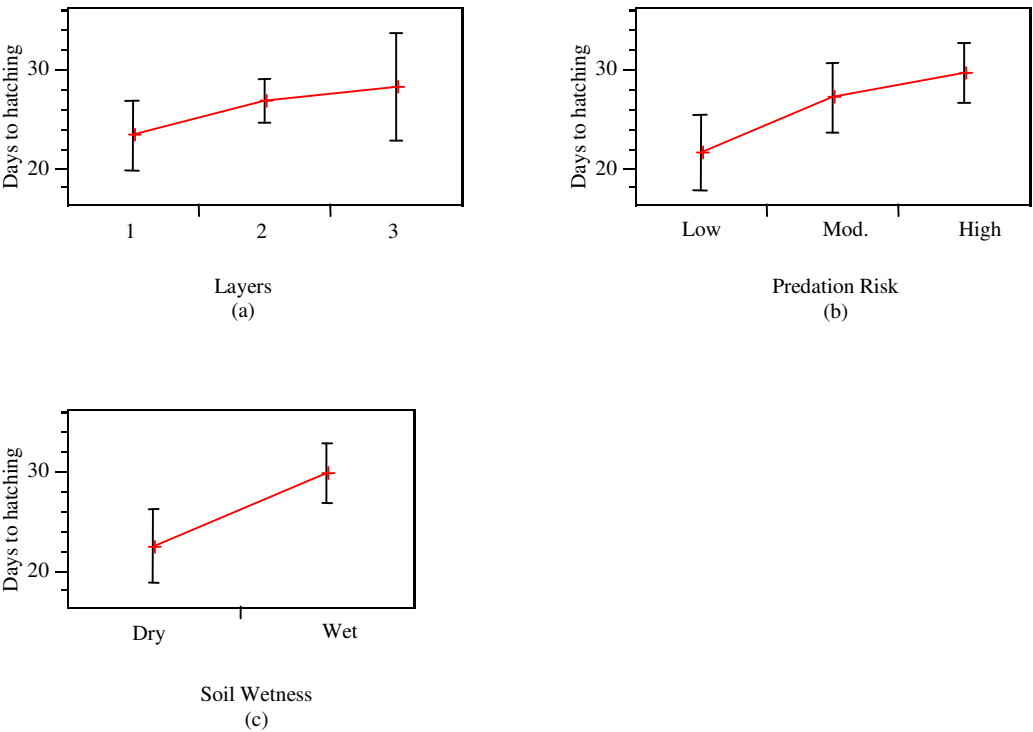


Fig. 4c. Quantiles plots after an ANCOVA analysis were egg cluster size and sun exposure (non-significant factors) were included. Days to hatching is affected is still affected by layering, predation risk, soil wetness of the host plant. Egg masses laid in one layer (a), on host plants with low predation risk (b) and on plants under dry soil condittions (c) develop faster.

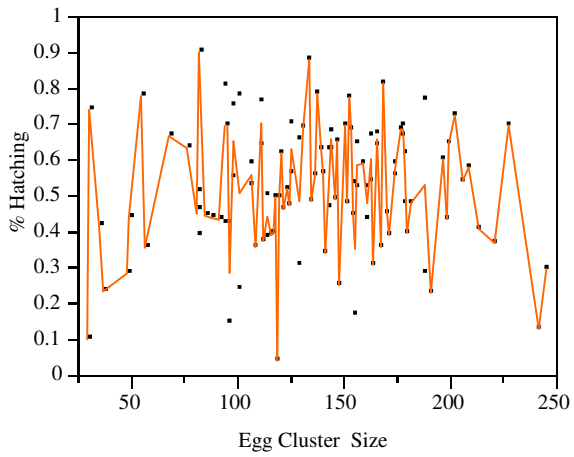


Figure 5. Scattered plot of ECS vs. % hatching with a line fitting the points. The points did not fit an expected bell shape curve. Therefore, there is no optimal egg cluster size that increases hatching success.